DNA sequence 1600 b.p.

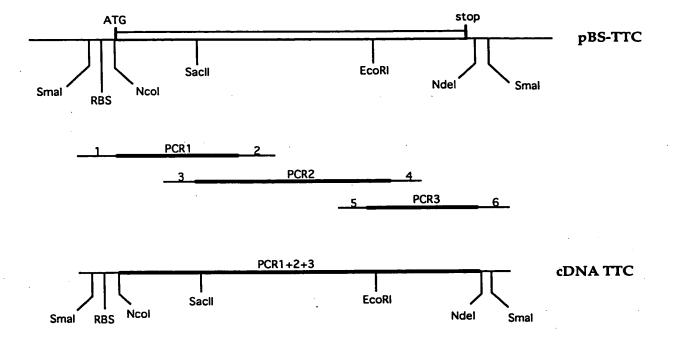
ggaaacagctat ... gtcgttttacaa linear

381 98 441 118 501 138 561 158 141 18 201 38 261 58 321 78 621 178 681 198 741 218 1 ggaaacagctatgaccatgattacgccaagctcgaaattaaccctcactaaagggaacaaaagctggagctcggtacccg TGG W GAT D TCT TTT F ည် ၁ GGT G GAA AAA K GGA TGT TTA L TAT Y GAA E AAT N \mathbf{r} TCT GGA G AAT N AAT N GAT AAT N AAT N AAT N ACA T CAA O AAT N GGA G GCG A GCA A ATA I GTA AAC TTA L CIG T ATA I TTT F GAT GAA E ATA I TAT Y TCC S GTA V TCT AAA AAT S K N ATT I ATG M TTA TCA S GAT D TTG L GAG E ACA T TTA TCT GAT CAT H GCT A CTA AAA K AGA R AGT S CAT H S CA AAT N AGT S TTA L AGT S AAT N GCT A ATT I $_{
m Y}^{
m TAT}$ AAG K AAT N TAT Y CAT H AAA GCA ATA K A I GCT A ACT T TTT F GCT A TCT AAA K TCT S TTT F GAA E TGG W TCT AAA K GGA G AAA K TCT $ext{TTT}$ TTA L 999 9 GAT D ATT I GTA V AAA K TTA L ATA I TTA L CCA P ATA AAT GGC I N G ATA I GAT D TTA ACT GGT T G TCT S AAA K CCT AGA R AGC TCT ATG S S M CCA ATT P I CCT P AAT AAC N GAT TTA D L GAT GTT V ATA I ATG M GAT D GAT D GCT GTT V AAT N ATT TCA ACA GGA ATA I TCA S AAA K ATT I AGG R AGG R GAA E GGT G ACT GAT D ATA I TTG GTG CCC L V P CAT H TTG ATA I AAA K A GCA TTT F ATT ATT TTT F GAA E GTG V S S TGG W ACT AGT S CTT ACT T GAT D GTT V GAA E ATA GGA G TTT F TAT Y S ATA I ATA I AAT N GTT V GAT GCT CAA D A Q 81 ggccacc ATG AAT N GAG E S CAA TTT F GTA V AGC S TCT GAA S E ACA AAT T N AAT N TGG AGT W S GAT D GTT V AGA R 202 ATT 2 39 I 142 GTT 19 V ACC GTT V 262 59 382 99 322 442 119 502 139 562 159 622 179 682 199

FIG. 1⊅

1221 378 1281 398 1535 464 1041 318 1101 338 1161 358 1341 418 1600 1401 438 981 298 1461 458 acagattgatatgttcatgacatatgcccgggatcctctagagtcgacctcgaggg SEQ ID NO:2) NO:1) TAT Y TAT Y GTT V GGT G AAT N ACC ACC TAC ATG M TTA L TTT F GTA V $_{\rm Y}^{\rm TAT}$ AAA K GGT G TGG W (SEQ ID AAA K TAT Y TCT ATT I AGG R TTA L GGT G AAC N AGT S GAT D AGA R GAT D CAC GAT D GTA V AGC S CTA ggggcccggtacccaattcgccctatagtgagtcgtattacaattcactggccgtcgttttacaa ACA T ATT ACA T TAT Y ATA I GAG E AGA R CGT R GCA A TCT ATA I TAT Y GAA E AAT N CTA TTG L TTA L ATT I GTT V AAT N AAT N AAT N ATA I ATT I AAA K TCT TTA L TAC TAC AAA K AAA K AAT N AAC N AAT N GTA V ATA I AGA R GCA A S CAA GAA TTG CCT TAT Y GAT GAT D GAT D GCA A AAT N AAT ATT I CCT CAA O AAA K ACA T TCA S GAA E AGG R CTT AAA K TGT AAT N TAT Y GTA V GTT V GGA G ATG M AAT N AAT N GAT D ୍ଦ୍ର ଓଷ୍ଟ AAT N AAA K AGA R GAT D AAC N $_{\rm Y}^{\rm TAT}$ AAA K CCA P AAT N GAT D AAT N AAA K TGG W AAA K ACT T TTA L TTT F AAA K GAT TAT Y TGT ATA I TCT S TAT Y AAA K TAT Y GCC TTA L AAC N AAA K AGA R TTA L GAC AGT S TCG S ATT I ATT I AAT N CTT AAA K ည္သ ဗ GAT GAT D TTT F TTT F TCT CCA P CCT GGA G CTT ATA I AAA K GAT D CTA GCG AAA K GAT D GCT GAT D ATC ₹° CAA AAT N AAA K CTA GTA V AAT N GGT G GGT G AAA K GTA V GGT G CAT H ACA T GGA G ACA TCA S CCG P CCA P AAT N AAT N ACA T ATA I ATA I TTG AAT N AAA K TAT Y CAT H gcc A TAT Y GGA G 862 259 1042 319 922 279 982 299 1102 339 1162 359 1222 379 1282 399 1536 1402 439

FIG. 1B



TTC cDNA isolation:

The TTC cDNA was isolated from a Clostridium Tetani strain using Polymerase Chain Reaction. We used a three times PCR to generate three overlapping fragments respectively of 465 bp (PCR1; primer 1:5'-CCC CCC GGG CCA CCA TGG TTT TTT CAA CAC CAA TTC CAT TTT CTT ATT C-3' (SEQ ID NO:4) & primer 2: 5'-CTA AAC CAG TAA TTT CTG-3'(SEQ ID NO:5), of 648 bp (PCR2; primer 3: 5'-AAT TAT GGA CTT TAA AAG ATT CCG C-3'(SEQ ID NO:6) & primer 4: 5'-GGC ATT ATA ACC TAC TCT TAG AAT-3'(SEQ ID NO:7) and of 338 bp (PCR3; primer 5: 5'-AAT GCC TTT AAT AAT CTT GAT AGA AAT-3'(SEQ ID NO:8) & primer 6: 5'-CCC CCC GGG CAT ATG TCA TGA ACA TAT CAA TCT GTT TAA TC-3'(SEQ ID NO:9), and each fragment was sequentially cloned into pBluescript KS+ to produce plasmid pBS-TTC. The upstream primer 1 contained the Ribosome Binding Site (RBS) and translation initiation signals.

FIG. 2

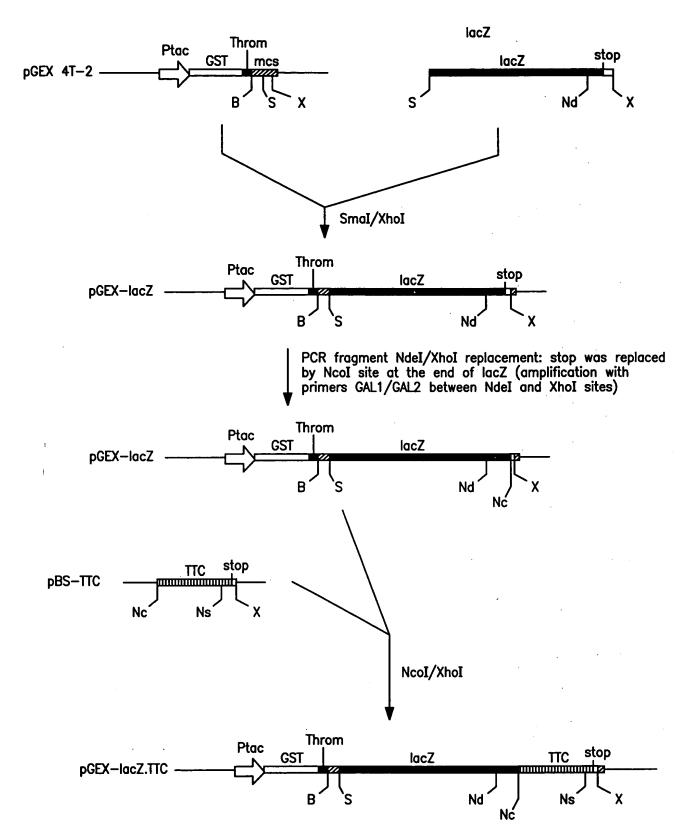


FIG. 3

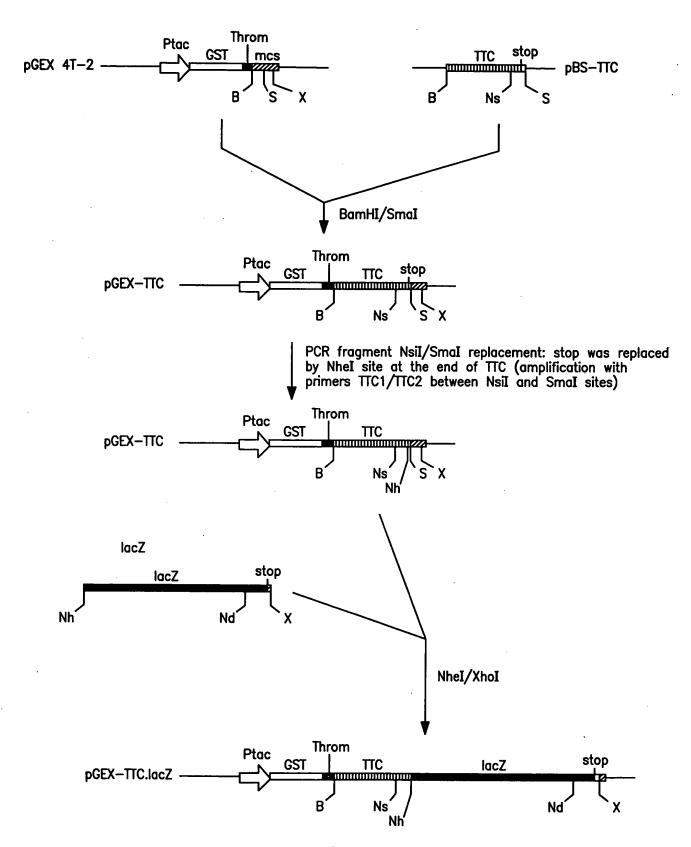


FIG. 4

pCMV.LACZ.TTC -> Graphic Map DNA sequence 8519 b.p.

tagttattaata ... accgccatgcat

circular

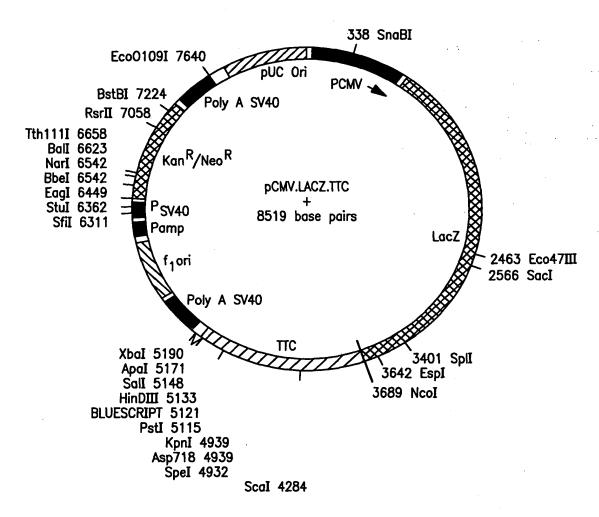


FIG. 5

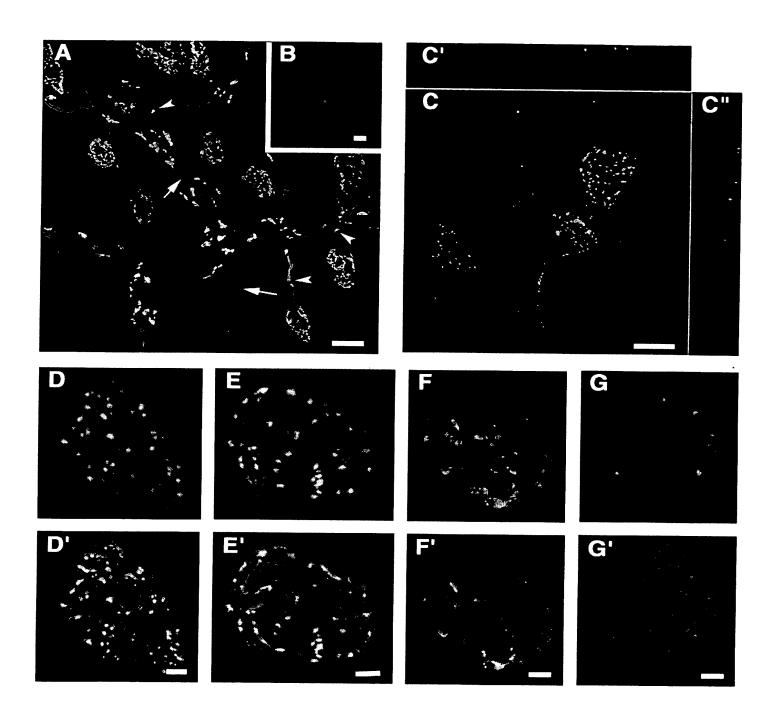
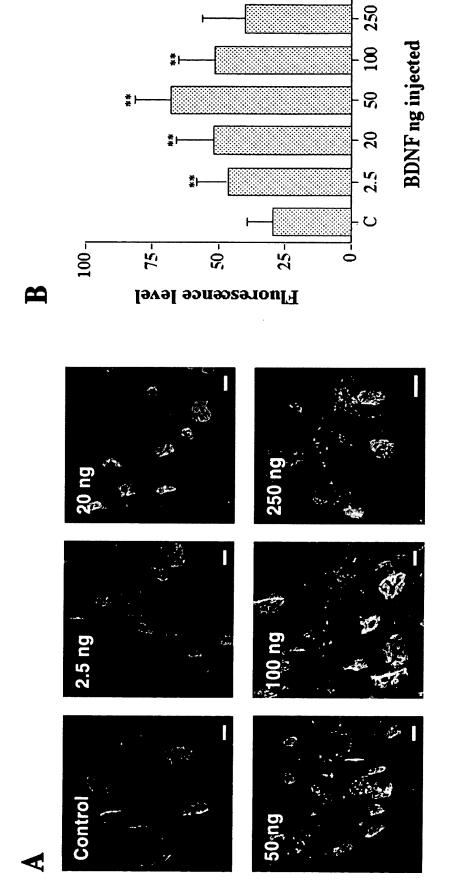


FIG. 6





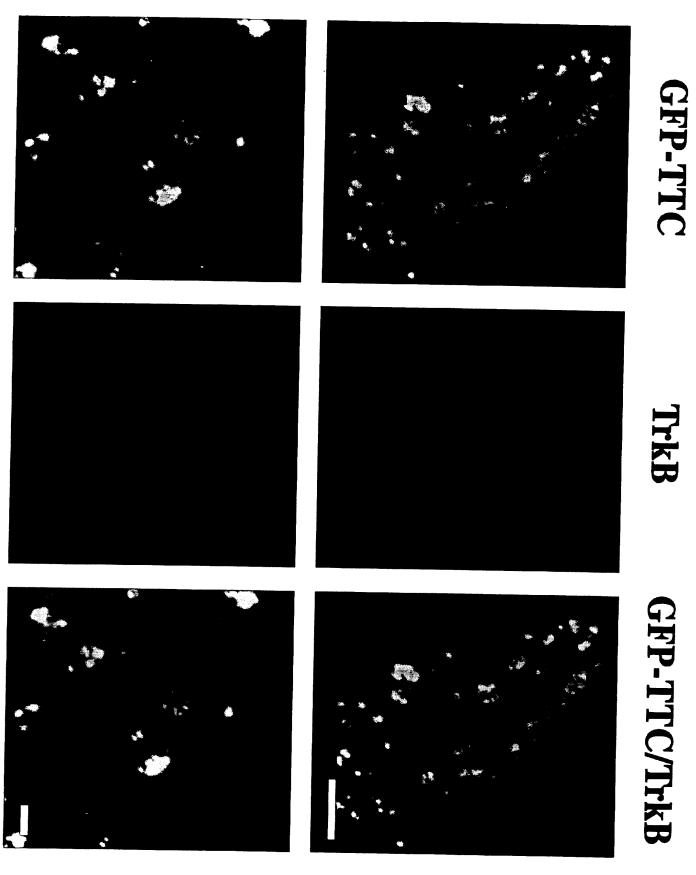
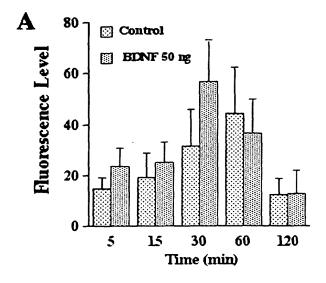
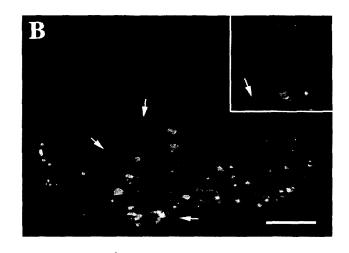
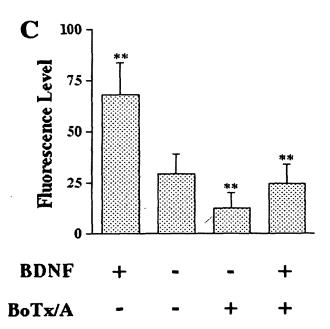


FIG. 8







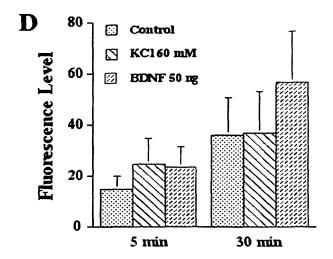


FIG. 9

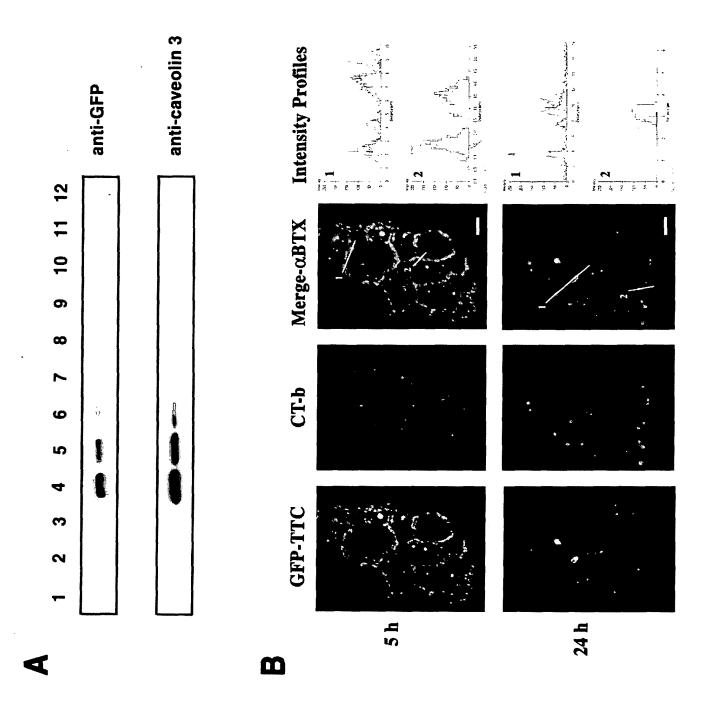


FIG. 10

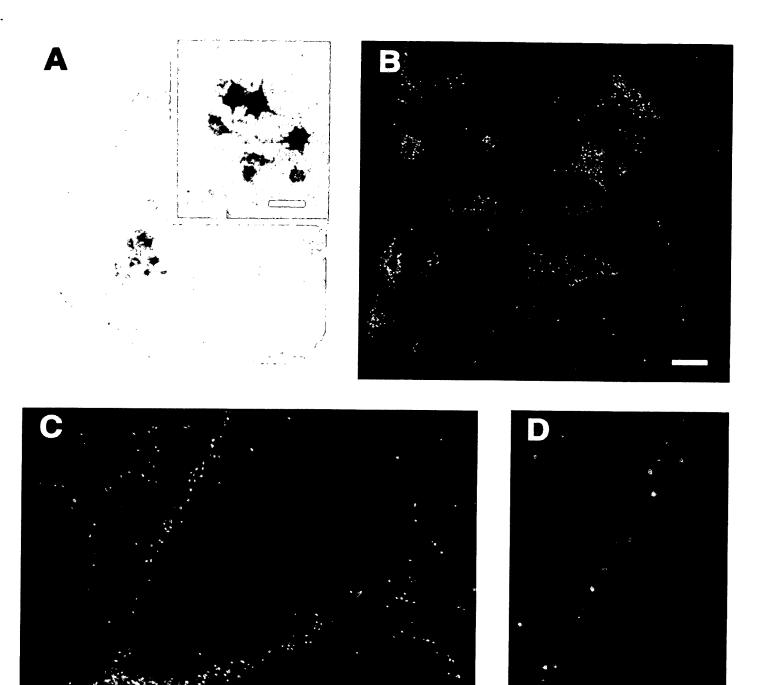


FIG. 11